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Online preconcentration in capillary electrophoresis with contactless conductivity detection for sensitive determination of sorbic and benzoic acids in soy sauce

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ABSTRACT

A sensitive method of online preconcentration followed by capillary electrophoresis with capacitively coupled contactless conductivity detection (CE-C^4D) is evaluated as a novel approach for the determination of benzoic acid and sorbic acid in soy sauce. The online preconcentration technique, namely field-enhanced sample injection, coupled with CE-C^4D were successfully developed and optimized. In order to reduce the complex matrix interference resulting from the constituents of soy sauce, a suitable sample clean-up procedure was also investigated for real sample pretreatment. Under optimized conditions, sorbic acid and benzoic acid were well separated within 10 min, and the detection limits were 0.05 μM (5.6 μg L^{-1}) and 0.08 μM (9.8 μg L^{-1}), respectively. The accuracy was tested by spiking 10.0 mg L^{-1} and 100.0 mg L^{-1} of standards in the soy sauce samples, and the recoveries were 95–99%, respectively. Results of this study show a great potential for the proposed method as a tool for the fast screening of benzoic acid and sorbic acid in a complex matrix.

1. Introduction

Sorbic acid and benzoic acid and its salts are important chemical preservatives used widely in pharmaceutical, cosmetic and food industry. They are added to delay nutritional losses of products due to germs and moulds during their shelf life, protecting consumer from the hazards of microbial toxins or pathogenic microorganisms. Benzoic acid inhibits bacterial development and sorbic acid is an antifungal preservative against moulds and yeasts [1,2]. The appropriate preservatives appear to be safe, which have been tested by many laboratories throughout the world [3,4]. However, individuals may be sensitive to various preservatives, so the kinds and the concentration of the preservative must be controlled.

Soy sauces contained considerable amount of sorbic acid, or benzoic acid, or both of them to stop germs and moulds from spoiling the nurture. Due to the complex matrix effect of the samples, sensitive and rapid simultaneous determination of sorbic acid and benzoic acid is required. The analyses of these compounds in food samples of differing matrices have been carried out by spectrophotometry [5], ion chromatography [6], gas chromatography (GC) [7], high-performance liquid chromatography (HPLC) [1,2,8–11], and capillary electrophoresis (CE) [4,12–16] in conjunction with a variety of detectors. Among those reports, CE has been considered as an efficient and cost-effective analytical method with many advantages, including minimum sample and reagent consumption, fast separation speed, and high theoretical plate number.

In CE, the recently developed capacitively coupled contactless conductivity detection (C^4D) has a number of advantages. It is universal for all ionic compounds without derivatization or indirect approaches [17–20]. The contactless conductivity detection features unprecedented ease of the cell arrangement and inherent prevention of the electrode fouling. Handing of the separation capillary is facile as it is not necessary to remove the polymeric cladding to create a detection window. Recent reviews on contactless conductivity detection are available [21,22].

Online preconcentration can be regarded as one of the major developments in CE specifically to overcome the sensitivity limitations of electrophoresis. This topic has attracted huge attention in the past 10 years and several reviews focusing on different online preconcentration techniques have been published even in the past 2 years [23,24]. Among the preconcentration techniques, field-enhanced sample injection (FESI) is most widely used and the simplest method to be performed, which is generally based on the electrophoretic migration of charged analytes between two different conductivity solutions. The sample is introduced elec-

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trokinetically, which allows more analytes to be injected into the capillary. This technique is well known due to its simplicity and high sensitivity enhancement with enrichment factors of several 100-fold or higher [25].

This report describes a simple, cost effective, and highly specific analytical method for the extraction, purification and quantitative determination of benzoic acid and sorbic acid in soy sauces by CE using field-amplified sample injection with capacitively coupled contactless conductivity detection (FESI-CE-C^4D). The performance of the method was evaluated with regard to the ability to generate accurate and precise qualitative and quantitative data in the relevant concentration range. To the best of our knowledge, this is the first study using FESI-CE-C^4D technique for the simultaneous determination of benzoic acid and sorbic acid in a complex matrix such as soy sauce samples.

2. Experimental

2.1. Apparatus

All experiments were performed on a laboratory-built CE unit, which comprised a ±30 kV high-voltage supply from Spellman High-voltage Electronics Co. (NY, USA), C^4D detector and digital data acquisition system assembled in-house. The C^4D excitation frequency was set to 350 kHz and the amplitude was boosted to 200 Vpp (peak-to-peak) using a purpose-made amplifier. The cell current was converted to a voltage, which was rectified, low pass filtered, and then digitized with the data acquisition system. Bare fused silica capillary of 50 μm i.d. and 375 μm o.d. from Hebei Ruifeng Instrumental Co. (Yongnian, Hebei, China) with a total and effective length of 45 cm and 40 cm, respectively, was employed.

2.2. Materials and chemicals

Sorbic acid and benzoic acid were obtained from J&K Chemical Co. (Beijing, China). HAc, Trihydroxymethyl aminomethane (Tris), Hexadecyl Trimethyl Ammonium Bromide (CTAB), were purchased from Guangzhou Chemical Reagent Co. (Guangzhou, China). All reagents were analytical grade unless otherwise indicated. Deionized water of at least 18 MΩ was required.

2.3. Solution preparation

Acetate acid, Tris and CTAB were dissolved in water respectively to prepare the concentrations of 0.2 M, 0.1 M and 0.01 M as the stock solution. Standard stock solution containing 500 mg L^{-1} sorbic acid and benzoic acid were prepared in 10% methanol aqueous solution, respectively. Stock solutions were stored in a 4 °C refrigerator and stable for at least 1 week. Working standard solutions of lower concentration were prepared by dilution with water prior to CE analysis. NaCl-saturated 6.0 M hydrochloric acid was prepared by adding solid sodium chloride into 6.0 M hydrochloric acid solution. The running buffer was prepared by mixing proper amounts of the stock solutions during the experiments.

2.4. Sample clean-up procedure

Real soy sauce samples were collected from the local supermarkets. The batch numbers (SS01, SS02, and SS03) were given based on three bands of soy sauces containing sorbic acid, or benzoic acid or both of them, respectively. An off-line liquid-phase extraction (LPE) step was used to clean-up the samples prior to FESI-CE-C^4D analysis. A 5.0 mL of soy sauces sample was filtered through a 0.45 μm cellulose ester membrane filters before the LPE. The percolate was collected, pH-adjusted to 1.5 with NaCl-saturated hydrochloric acid. A 1.0 mL of sample solution was then added in a 10 mL centrifuge tube with glass stopper, followed by another 5.0 mL of anhydrous diethyl ether. The sample solution was ultrasonically extracted for 5 min and refrigerated centrifuged at 3000 rpm for 2 min. Then, A 3.0 mL of the organic phase solution was washed with 3.0 mL NaCl-saturated hydrochloric acid and kept still for 15 min. Subsequently, A 1.0 mL of the organic phase solution was evaporated to dryness in a stream of nitrogen gas. The residue was dissolved in 1.0 mL 35 μM Tris solution (see Section 3.2.1). Finally, an appropriate diluted sample solution was applied to FESI-CE-C^4D analysis.

2.5. Electrophoresis procedures

Bare capillary was conditioned by washing with 0.1 M NaOH, water and running buffer for 5 min, respectively. The anodic and cathodic reservoirs were ultrasonically rinsed in water. Whenever the running buffer was altered, the above steps were repeated. After each analysis run, the capillary was rinsed for 3 min with the running buffer to maintain the reproducibility of the analysis. LODs were determined corresponding to peak area for S/N of 3. Peak identification was performed by standard addition method. The experiment was done under the required laboratory environment with constant temperature (25 °C) and low humidity (<60%).

3. Results and discussion

3.1. Choice of BEG solution and separation voltage

The background electrolyte (BGE) affects the migration time and the separation between compounds directly. In the selection of BGE, the main consideration is the ionization characteristic of the analytes. The p*K*a values for benzoic acid and sorbic acid are 4.20, 4.76, respectively [26], which indicated that the analytes have a net negative charge in an aqueous BGE when pH > 4.5. Therefore, in our study, negative polarity separation voltage was used. However, in negative polarity separation voltage mode, the direction of the electroosmotic flow (EOF) was opposite to that of anion electromigration resulted in very poor resolution and detection capability when pH > 4.5, it is favorable to use the EOF modifier to suppress or reverse EOF direction. In our study, 0.2 mM CTAB was used as the EOF modifier. Several electrolytes utilized as the buffer solution were tested, including HAc, NaAc, MES, Tris, His, and Arg. Among them, HAc/Tris provided satisfactory results with the highest sensitivity response relative to the others. Thus, a combination of HAc/Tris was used as the BGE. The effect of the pH of the BGEs was studied. The pH changed from 4.0 to 8.0 by adjusting the ratio of HAc and Tris. The highest sensitivity responses for the both analytes were achieved when 20 mM HAc/8 mM Tris (pH = 4.8) was utilized as separation BGE.

The efficiency of the separation improves and the migration time shortens when the separation voltages is increased. As known, higher voltage will result in the peak broadening because of the Joule heating effect, and the resolutions between benzoic acid and sorbic acid became worse. In our study, a voltage of −15 kV was selected as the best separation voltage.

3.2. Optimization of FESI conditions

In FESI, the conductivity of the sample solution is lower than that of BGE. Theoretically, the amount of stacking is proportional to the conductivity difference between the running buffer and the sample solution [27]. In practice, the analytes usually dissolved in water or diluted buffer solution, which leads to a narrow higher concentration sample zone [23]. In order to achieve the maximum amount of analytes loaded into the capillary, several parameters such as sample solution composition, the water plug length, injection voltage,

and injection time must be optimized in order to obtain the best FESI efficiency.

3.2.1. Effect of sample solvent composition

In general, the amount of sample injected electrokinetically could be increased when the analytes possess higher mobility. This mobility was greatly affected by the dielectric constants and viscosity of the sample solvent used [28]. It has been showed that high enhancement in FESI can be achieved easily by dissolving the sample in a low conductivity solvent such as water, ten times lower concentration of running buffer or in low proportions of organic solvent in water [29]. Several solvents were tested in our study which containing water, diluted buffer solution and low concentration Tris solution. The best result was obtained when the analytes were dissolved in 35 μM Tris solution. Thus, the analytes was prepared in 35 μM Tris solution to enhance compound ionization and improve reproducibility for method optimization.

3.2.2. Effect of water plug injection

Several reports had proved that the utilization of a water plug in FESI can greatly increase the reproducibility of the method [30–32]. By injecting a short water plug prior to electrokinetic sample introduction, a higher electric field at the injection point will be generated, thus ensuring a higher capability in concentrating the charged analytes into the capillary and leading them away from the inlet end. In our study, the hydrodynamic injection of a water plug in the range of 0–9 s with 3 s increments was examined. However, no improvement of peak intensities for the analytes was observed when water plug was introduced. This phenomenon indicated that the direction of the EOF and electrophoretic mobility of the analyte ions are the same, and the water plug was not very necessary when the EOF is directed toward the detector [33,34]. Thus, in order to simplify the procedure, water plug injection was not used in our study.

3.2.3. Effect of injection voltage and injection time

The injection voltage and injection time are the most crucial factors that affect the sensitivity enhancement in FESI. A series of injection voltages ranging from −5 kV to −15 kV were tested in our study. Significant increase of peak intensity was noted when the injection voltage was increased. However, a minor peak-broadening was observed for the analytes when the injection voltage reached −12 kV. The peak broadening problem in conjunction with peak distortion became more obvious when the injection voltage was increased to −15 kV. Thus, an injection voltage of −12 kV was chosen as the optimum injection voltage. Optimization of injection time was carried out by injecting the sample electrokinetically in the range of 6–18 s with 3 s increments at −12 kV. A marked increase of peak intensity was observed when the injection time was increased from 6 s to 12 s. A further increase of injection time to 18 s resulted in a serious peak broadening problem. Therefore, an injection time of 10 s was chosen as the optimum injection time.

Based on the results shown above, the optimum conditions of FESI were selected as follows: sample solvent, 35 μM Tris solution; electrokinetic injection voltage, −12 kV; electrokinetic injection time, 10 s.

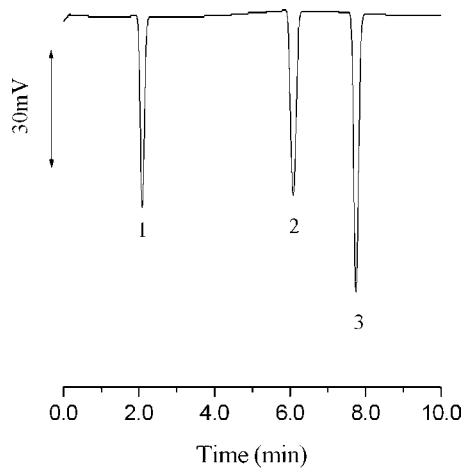

Fig. 1. Electropherogram of a mixture containing 3.0 μM of sorbic acid and benzoic acid with FESI-CE-C^{4}D. Conditions: 20 mmol/L HAc + 6 mmol/L Tris + 0.2 mmol/L CTAB as running buffer; electrokinetic injection, −12 kV × 10 s; separation voltage, −15 kV; fused-silica capillary, 50 μm × 45 cm (40 cm to detector); sample dilution solvent, 35 μM Tris solution. Peak identification: 1, unknown; 2, benzoic acid; 3, sorbic acid.

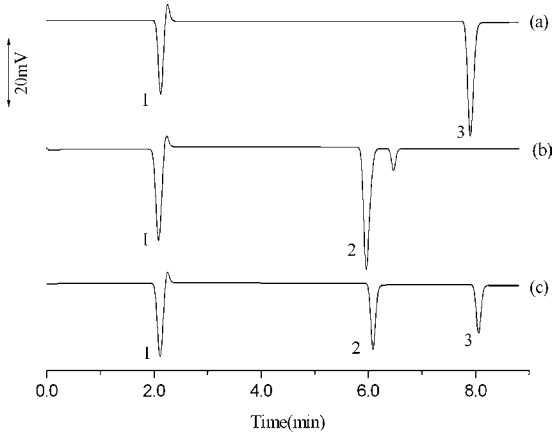

Fig. 2. Electropherograms for the separation of sorbic acid and benzoic acid in real soy sauce samples. (a) SS01; (b) SS02; (c) SS03. Other conditions and peak identification are the same as mentioned in Fig. 1.

3.3. Linearity, detection limits, and precision

Under the optimum FESI-CE-C^{4}D condition, the typical electropherogram for a mixture containing 3.0 μM of sorbic acid and benzoic acid was shown in Fig. 1. The method validation including linearity, limits of detection (LOD), and precision was carried out, and were summarized in Table 1. There was an excellent linearity between the peak area (mV s) and the concentration (μM) of sorbic acid and benzoic acid in the range of 0.3–20.0 μM, with the corre-

Table 1
The results of linearity, limits of detection (LOD), and precision.

Compounds	Linear regression[a]	Linearity (μM)	Correlation (R, n = 6)	LOD[b] (μM)	LOD (μg L^{-1})	RSD% (n = 6)
Sorbic acid	y = 594.9x + 9.3	0.3–20.0	0.9993	0.05	5.6	3.5[c], 3.8[d]
Benzoic acid	y = 482.3x + 8.2	0.3–20.0	0.9993	0.08	9.8	3.5, 3.6

[a] Linear regression based on peak area (mV s) vs. concentration (μM).
[b] Estimated on the basis of S/N = 3.
[c] The run-to-run precision.
[d] The day-to-day precision.

Table 2
Assay results of benzoic acid and sorbic acid in soy sauce samples[a] compared with HPLC.

Sample	Sorbic acid (mg L^{-1})		Benzoic acid (mg L^{-1})	
	FESI-CE-C^{4}D	HPLC	FESI-CE-C^{4}D	HPLC
SS01	214 (3.9)[b]	218 (1.9)	ND	ND
SS02	ND	ND	273 (3.9)	268 (1.8)
SS03	140 (3.8)	136 (2.1)	82 (3.8)	80 (2.2)

ND: not detected.
[a] Conditions are the same as in Fig. 1.
[b] The data in the brackets are the RSD% (n = 3).

Table 3
Recovery of spiked standard in real soy sauce samples[a].

Compound	Init. amount[b] (mg L^{-1})	Added (mg L^{-1})	Found (mg L^{-1})	Recovery (%)
Sorbic acid	214	100.0	311	99.0
	0	10.0	9.5	95.0
Benzoic acid	273	100.0	369	98.9
	0	10.0	9.6	96.0

[a] CE conditions are the same as in Fig. 1.
[b] The initial amount of benzoic acid and sorbic acid in original soy sauce sample.

lation coefficients r = 0.9993. The LODs were 0.05 μM (5.6 μg L^{-1}) and 0.08 μM (9.8 μg L^{-1}), respectively. The run-to-run and day-to-day precision was tested at 3.0 μM levels, RSDs were found to be lower than 5.0% (n = 6), indicating good repeatability.

3.4. Applications and recoveries

Three bands of real soy sauce samples with the batch numbers (SS01, SS02, and SS03) were treated with the procedure depicted in Section 2.4 prior to FESI-CE-C^{4}D analysis. Fig. 2 demonstrated the electropherograms. The determination results of sorbic acid and benzoic acid in the samples are listed in Table 2. The results confirm that SS01 and SS02 contain sorbic acid or benzoic acid only, while both of sorbic acid or benzoic acid were found in SS03, which were in accord with the label ingredients. The determination results of benzoic acid and sorbic acid in real soy samples were lower than the maximum addition levels established by China [35]. The quantitative results were further evaluated by HPLC method (data shown in Table 2). The significance testing results revealed that the quantitative results of the proposed method were reliable. Compared with HPLC method, FESI-CE-C^{4}D method is better for its simplicity in operation and low consumption of organic solvent. The assay indicated that the analytical method proposed shows great potential in the determination of benzoic acid and sorbic acid in soy sauces.

Recovery experiments were performed by adding accurate amounts of sorbic acid and benzoic acid to the real soy sauce samples. The standard-spiked samples were subject to the sample preparation procedure depicted in Section 2.4 followed by the FESI-CE-C^{4}D analysis. The resulting recovery values were summarized in Table 3. Satisfactory spiked recoveries show that the proposed FESI-CE-C^{4}D method was suitable to be utilized for the determination of benzoic acid and sorbic acid in a complex matrix such as soy sauce samples.

4. Conclusions

A sensitive method for the simultaneous determination of sorbic acid and benzoic acid in soy sauces samples by FESI-CE-C^{4}D has been developed. The FESI-CE-C^{4}D technique is advantageous in terms of simplicity, cost effectiveness, high sensitivity, as well as excellent repeatability. For the first time, to our knowledge, FESI-CE-C^{4}D coupling a sample clean-up method for the analysis of sorbic acid and benzoic acid in a complex matrix has been comprehensively optimized. Excellent detection limits were obtained for sorbic acid and benzoic acid. The applicability of the developed methods to the real soy sauce samples analysis demonstrated satisfactory recoveries and reproducibility. The current results provide a great incentive to further investigate the applicability of FESI-CE-C^{4}D method in order to achieve detection limits below the legislated maximum concentration limits.

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